Targeting CD8$^+$ T cells prevents psoriasis development

To the Editor:

In psoriasis, intraepidermal T cells are predominantly CD8$^+$ and represent key effector cells. Here, we show that these T cells produce pathogenic IL-17 and that neutralization of CD8$^+$ T cells effectively prevents psoriasis development *in vivo*.

Psoriasis is a common inflammatory skin disease, resulting from the interaction of genetic and environmental triggers, leading to dysregulated immune response of innate and adaptive immune cells.[1,2] T lymphocytes infiltrating psoriasis skin lesions play key effector roles by driving disease development and maintenance. Traditionally, CD4$^+$ T_H cells producing proinflammatory cytokines, such as IL-17A, IL-22, and IFN-γ, are regarded as the main pathogenic T-cell subpopulation. However, CD8$^+$ T cells, which are present in healthy skin as tissue resident memory T cells (T_{RM}),[3] have been shown to produce a similar profile of proinflammatory cytokines[4]; they are abundantly present in the psoriatic epidermis and potentially recognize peptide antigens presented on MHC class I molecules, such as HLACw6, which is the strongest psoriasis susceptibility allele.[5] Furthermore, we have previously shown that intraepidermal T cells represent key effector cells in psoriasis development and that impeding the entry of T cells into the epidermis, by blocking α1β1-integrin, prevents the development of psoriasis in the clinically relevant AGR mouse model of psoriasis.[6] Thus, we set out to explore the pathogenic relevance of CD8$^+$ T cells in psoriasis.

We first performed a time course experiment using the AGR mouse model. AGR mice are grafted with noninvolved skin from patients with psoriasis, which spontaneously develops a psoriatic phenotype after 4 to 6 weeks.[6] Thus, at days 0, 7, 21, and 35, skin transplants were harvested and processed for histological and immunohistochemical assessment as described previously.[6] In line with earlier findings, while the proliferation of dermal T cells preceded epidermal changes, the numerical expansion of the epidermal T-cell pool temporally coincided with the onset of the psoriatic phenotype, as shown by the papillomatosis index

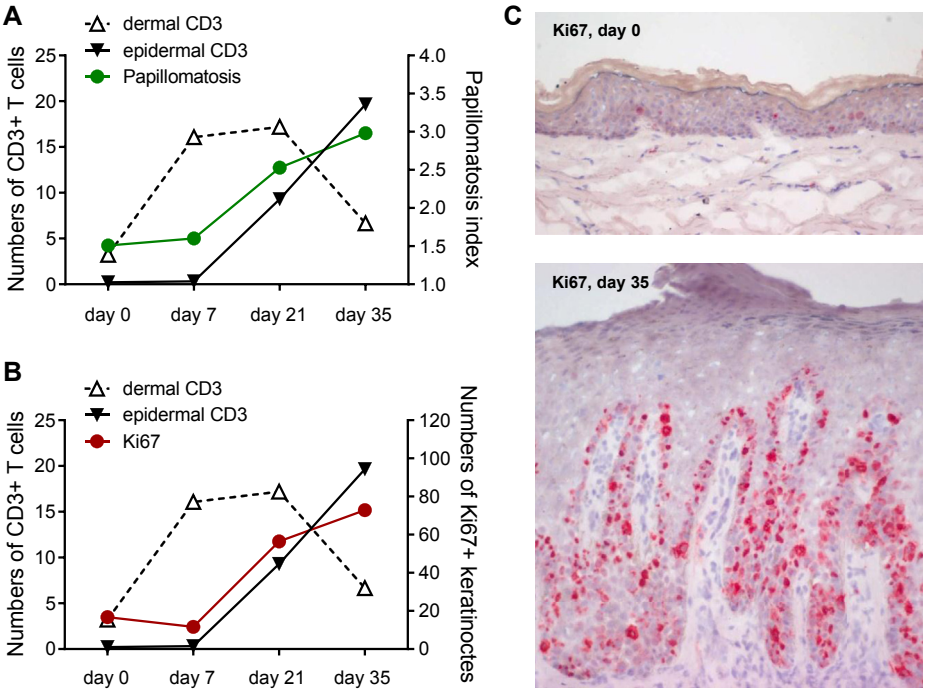

FIG 1. Expansion of epidermal T cells induces epidermal hyperproliferation and onset of a psoriatic phenotype. Quantification of T-cell numbers during psoriasis development in the AGR mouse model (days 0, 7, 21, and 35): dermal (*dashed black line*, **A** and **B**) and epidermal (*solid black line*, *A* and *B*) T-cell counts compared with papillomatosis index (*green line*, *A*) and number of Ki67-positive keratinocytes per 100 basal keratinocytes (*red line*, *B*) during psoriasis development. Microscopic view of nonlesional psoriatic skin stained with an mAb to Ki-67 on the day of transplantation onto AGR mice and after development of fully fledged psoriasis on day 35 **(C)**. Data depicted correspond to mean values and reflect 1 representative experiment of 2 independent experiments with skin from 2 donors (n = 3-4 transplanted mice for every time point). Values of standard error of the mean (SEM) for each parameter and time point are depicted in Table E1 in this article's Online Repository at www.jacionline.org.

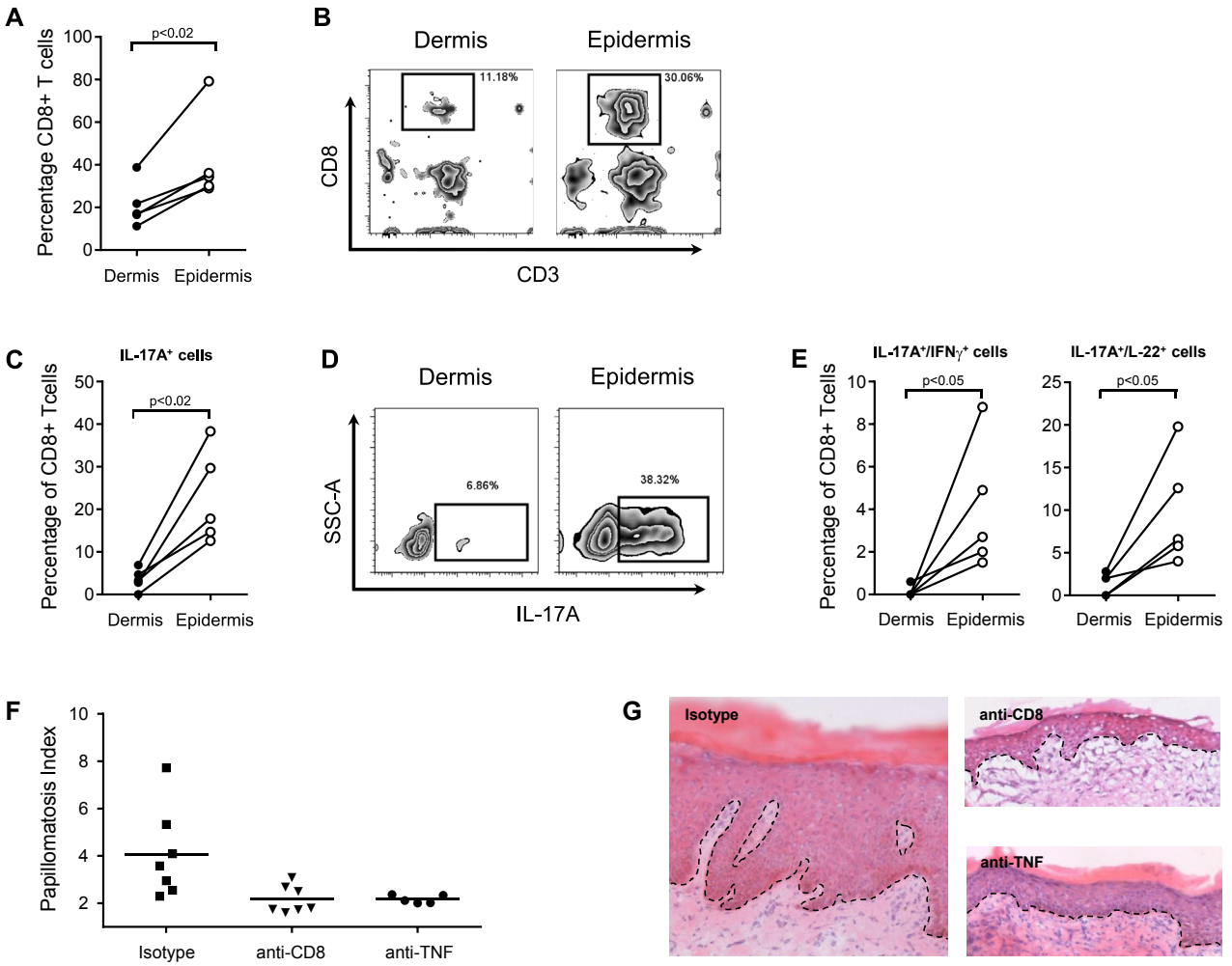

FIG 2. Epidermal $CD8^+$ T cells are highly pathogenic and their blockade prevents the development of psoriasis. Relative frequency **(A)** and representative zebra plot **(B)** of $CD3^+CD8^+$ T cells among live $CD45^+$ cells isolated from the epidermis and the dermis of 5 patients with psoriasis, with each patient denoted by a connecting line. Functional characterization of epidermal and dermal $CD3^+CD8^+$ T cells as $IL\text{-}17A^+$ **(C)**, with representative zebra plot in **(D)** $IL\text{-}17A^+IFN\text{-}\gamma+$ and $IL\text{-}17A^+IL\text{-}22^+$ **(E)** obtained by intracellular cytokine staining after phorbol 12-myristate 13-acetate/ionomycin stimulation. Each line connects epidermal and dermal $CD8^+$ T cells from the same patient. **F,** Microscopic changes of nonlesional psoriatic skin quantified using the papillomatosis index 35 days after transplantation onto AGR mice treated with isotype control mAb (mean ± SEM, 4.07 ± 0.72), mAb to CD8 (2.17 ± 0.22), or anti-TNF mAb (2.17 ± 0.08). **G,** Representative microscopic views of nonlesional psoriatic skin 35 days after transplantation onto AGR mice treated with isotype control mAb, mAb to CD8, or anti-TNF mAb. *B* and *D*, Zebra plot shown is representative of 1 of 5 patients. *F*, Data shown represent pooled results from 2 independent experiments with skin of 2 patients. Each symbol represents a transplanted mouse (n = 5-7). Statistical analyses in *A*, *C*, and *E* were performed by paired *t* test and in *F* with ANOVA followed by Bonferroni correction. All testing was 2-sided, and a *P* value of less than .05 was considered to indicate statistical significance.

(Fig 1, *A* and *C*, and Conrad et al[6]). Moreover, the accumulation of epidermal T cells paralleled the increase in proliferating keratinocytes as identified by positive Ki67-staining (Fig 1, *B* and *C*). Importantly, in the absence of T-cell expansion upon transplantation, which did not occur in one of the experiments we performed, we did not observe any epidermal pathology, in terms of both papillomatosis and frequency of proliferating keratinocytes (see Fig E1 in this article's Online Repository at www.jacionline.org). Thus, the accumulation of epidermal T cells induces both keratinocyte hyperproliferation and onset of papillomatosis, 2 hallmarks of psoriasis, thereby further confirming the role of intraepidermal T cells as key effectors in psoriasis.

In keeping with the classical distribution of $CD4^+$ and $CD8^+$ T cells in human psoriatic lesions, intraepidermal T cells in skin grafts 35 days posttransplant were predominantly $CD8^+$ T cells (see Fig E2 in this article's Online Repository at www.jacionline.org). Psoriatic $CD8^+$ T cells have been previously characterized in terms of their cytokine production; however, little distinction has been made between those residing in the dermis and the epidermis in the absence of post-isolation *in vitro* culture. Thus, to obtain a faithful functional characterization of psoriatic $CD8^+$ T cells, we isolated T cells from the epidermis and the dermis of psoriasis lesions and performed intracellular cytokine staining and fluorescence-activated cell sorting analyses. Among

live CD45$^+$ immune cells, the frequency of CD3$^+$CD8$^+$ T cells was significantly higher in the epidermis than in the dermis (Fig 2, *A* and *B*). Interestingly, the frequency of epidermal CD3$^+$CD8$^+$ T cells producing IL17A (Fig 2, *C* and *D*) or double-producing both IFN-γ and IL-17A or IL-22 and IL-17A, respectively (Fig 2, *E*), significantly exceeded that of dermal CD3$^+$CD8$^+$ T cells. No significant difference was found for IFN-γ$^+$, IL-22$^+$, or IL-22$^+$IFN-γ$^+$ T cells between the dermis and the epidermis (see Fig E3 in this article's Online Repository at www.jacionline.org). Thus, the main factor differentiating the epidermal from the dermal CD8$^+$ T-cell population is an active Tc17 phenotype.

On the basis of these findings, we sought to determine the *in vivo* pathogenic relevance of CD8$^+$ T cells infiltrating psoriasis lesions. Therefore, we treated xenotransplanted mice with either 1 mg mAb to human CD8 (M-T807) or the corresponding isotype control mAb on days 0 and 14, or mAb to TNF (infliximab, 1 mg intravenously on days 7 and 21 after transplantation). Isotype control antibody-treated skin grafts developed fully fledged psoriasis over the course of 35 days (Fig 2, *F* and *G*). Injection of mAb to CD8 resulted in a significantly reduced papillomatosis index and complete blockade of psoriasis development. The effect was equivalent to that obtained with TNF antagonists, a current benchmark in psoriasis treatment (Fig 2, *F* and *G*).

CD8$^+$ T cells and their role in psoriasis are currently under the spotlight (see Fig E4 in this article's Online Repository at www.jacionline.org). CD8$^+$ T cells isolated from patients with psoriasis produce psoriasis-relevant cytokines, they are retained in the epidermis as T_{RM} after successful therapy,[7] and LL-37–specific CD8$^+$ T cells expressing α1β1-integrin, a key molecule for trafficking of T cells into psoriatic epidermis,[6] have been identified in psoriatic blood.[8] The preferential anatomical location in the epidermis makes CD8$^+$ T cells ideally located to engage in a pathogenic cross talk with keratinocytes (Fig E4); a recent mouse model of psoriasiform murine inflammation relying on keratinocyte genetic abnormalities identified CD8$^+$ T cells as critical players.[9] In addition, we show that the accumulation of epidermal T cells, which mainly reflect CD8$^+$ T cells, correlates with the onset of keratinocyte hyperproliferation and papillomatosis, 2 characteristic features of psoriasis. Epidermal CD8$^+$ T cells display highly pathogenic features, and the significantly increased frequency of those producing IL-17A, alone or in combination with IL-22 and IFN-γ, makes them a reasonable primary source for this pivotal cytokine, whose clinical targeting is proving highly successful.[10] Finally, we show that blockade of CD8$^+$ T cells via a neutralizing mAb prevents the development of psoriasis in a clinically relevant xenotransplantation mouse model, thus uncovering a critical role for them in driving pathology. These findings may provide the basis for the design of new strategies targeting CD8$^+$ T cells for the treatment of psoriasis.

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REFERENCES

1. Di Meglio P, Villanova F, Nestle FO. Psoriasis. Cold Spring Harb Perspect Med 2014;4.
2. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol 2014;32:227-55.
3. Watanabe R, Gehad A, Yang C, Scott LL, Teague JE, Schlapbach C, et al. Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. Sci Transl Med 2015;7:279ra39.
4. Hijnen D, Knol EF, Gent YY, Giovannone B, Beijn SJ, Kupper TS, et al. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. J Invest Dermatol 2013;133:973-9.
5. Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012;44:1341-8.
6. Conrad C, Boyman O, Tonel G, Tun-Kyi A, Laggner U, de Fougerolles A, et al. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. Nat Med 2007;13:836-42.
7. Cheuk S, Wiken M, Blomqvist L, Nylen S, Talme T, Stahle M, et al. Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. J Immunol 2014;192:3111-20.
8. Lande R, Botti E, Jandus C, Dojcinovic D, Fanelli G, Conrad C, et al. The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis. Nat Commun 2014;5:5621.
9. Gunderson AJ, Mohammed J, Horvath FJ, Podolsky MA, Anderson CR, Glick AB. CD8(+) T cells mediate RAS-induced psoriasis-like skin inflammation through IFN-gamma. J Invest Dermatol 2013;133:955-63.
10. Langley RG, Elewski BE, Lebwohl M, Reich K, Griffiths CE, Papp K, et al. Secukinumab in plaque psoriasis–results of two phase 3 trials. N Engl J Med 2014;371:326-38.

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Human nasal epithelial cells derived from multiple subjects exhibit differential responses to H3N2 influenza virus infection *in vitro*

To the Editor:

Nasal epithelium is the first line of mechanical and immunologic defense in the upper respiratory tract.[1] Upper respiratory

METHODS

Animal studies were approved by the Kantonale Veterinaeramt of Zurich. Human studies, conducted according to the Declaration of Helsinki, were approved by the institutional review boards of the University Hospital of Zurich and Guy's and St Thomas' Hospital and informed patient consents were obtained. Xenotransplantation of noninvolved psoriatic skin, obtained from 3 patients with psoriasis, was performed as previously described using AGR129 mice, which are deficient in type I (A) and type II (G) IFN receptors in addition to being *Rag2*$^{-/-}$ (R). After 4 to 6 weeks, these skin grafts spontaneously develop a psoriatic phenotype including thickening of the epidermis (acanthosis), elongation of the rete ridges (papillomatosis), and increased numbers of dermal and epidermal T cells, closely reflecting the pathology of patient samples.[E1] For fluorescence-activated cell sorting analyses of skin T cells, we obtained 4-mm full-thickness skin biopsies from 5 patients with psoriasis, incubated them in 0.5 mol/L EDTA at 37°C for 3 hours to separate the epidermis and the dermis, and then digested them separately in 0.8 mg/mL collagenase type IV in RPMI + 10%FCS + 1%Pen/Strep (cRPMI) at 4°C overnight. EDTA treatment does not affect the expression of cell surface markers, such as CD4, in contrast to the widely used dispase treatment (data not shown). Subsequently, digested tissue was stimulated with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomicin (1 μg/mL) in the presence of brefeldin A (3 μM) and monensin (3 μM) in cRPMI at 37°C for 5 hours. Dead cells were excluded from the analysis by staining with Live Dead Yellow (Life Technologies, Carlsbad, Calif). Cells were stained for surface markers, fixed and permeabilized, and stained for intracellular cytokines. The following antibodies were used: anti-CD3 APC (SK7, BD Biosciences, Franklin Lakes, NJ), anti-CD4 BV650 (SK3, BD Biosciences), anti-CD8 PE-Texas Red (3B5, Invitrogen, Carlsbad, Calif), anti-CD45 V500 (HI30, BD Biosciences), anti–IL-17A V450 (N49-653, BD Biosciences), anti–IL-22 PerCP-eFluor710 (22URTI, eBiosciences, San Diego, Calif), and anti–IFN-γ A700 (B27, BD Biosciences).

REFERENCE

E1. Conrad C, Boyman O, Tonel G, Tun-Kyi A, Laggner U, de Fougerolles A, et al. Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. Nat Med 2007;13:836-42.

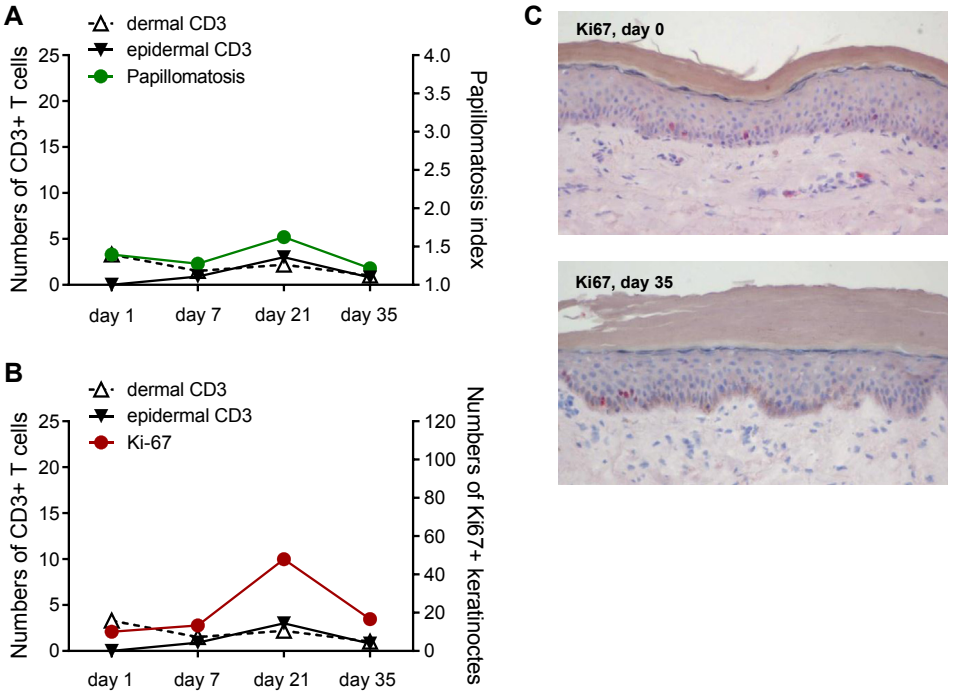

FIG E1. Absence of epidermal pathology after failed T-cell expansion. Quantification of T cells present in skin samples upon transplantation in the AGR mouse model (days 0, 7, 21, and 35): dermal (*dashed black line*) and epidermal (*solid black line*) T-cell counts compared with papillomatosis index (*solid green line*, **A**) and Ki-67 postive keratinocytes (*solid red line*, **B**) during psoriasis development. Microscopic view of nonlesional psoriatic skin stained with an mAb to Ki-67 on the day of transplantation onto AGR mice and on day 35 **(C)**. Data in *A* and *B* reflect 1 experiment with skin from a single donor not showing any relevant T-cell proliferation upon transplantation (n = 2 for every time point).

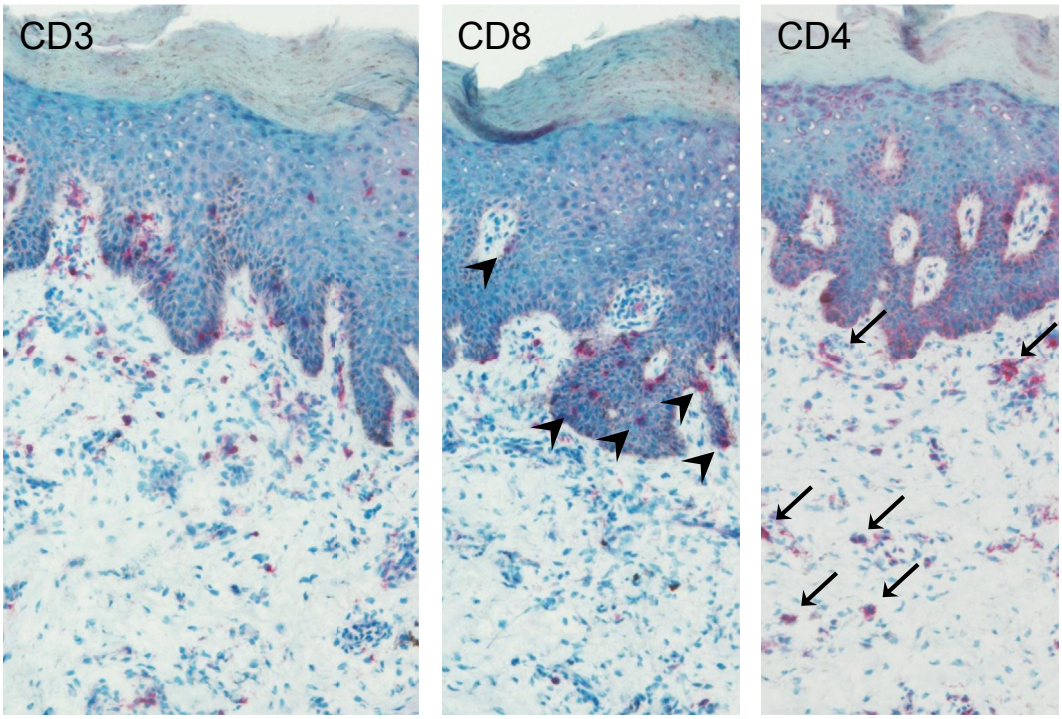

FIG E2. As in classical human psoriasis, intraepidermal T cells in the AGR mouse model represent mostly $CD8^+$ T cells. Microscopic view of representative CD3, CD4, and CD8 immunostaining of nonlesional psoriatic skin upon development of fully fledged psoriasis, 35 days after engraftment onto AGR mice. *Arrowheads* depict intraepidermal $CD8^+$ T cells, and *arrows* depict dermal $CD4^+$ T cells.

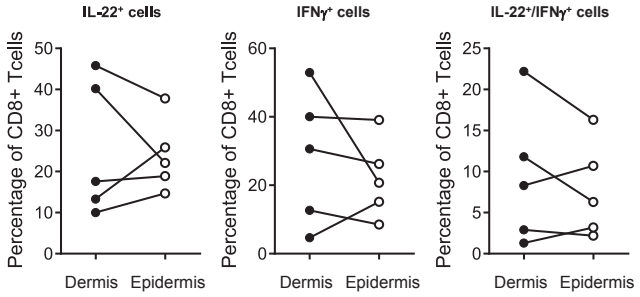

FIG E3. No differences in IL-22 and IFN-γ production between epidermal and dermal $CD8^+$ T cells. Functional characterization of epidermal and dermal $CD3^+$ $CD8^+$ T cells isolated from the epidermis and the dermis of 5 patients with psoriasis. Percentages of $IL\text{-}22^+$, $IFN\text{-}\gamma^+$, and $IL\text{-}22^+$ $IFN\text{-}\gamma^+$ $CD8^+$ T cells, as obtained by intracellular cytokine staining upon phorbol 12-myristate 13-acetate/ionomycin stimulation, show no differences between dermal and epidermal $CD8^+$ T cells. Each *line* connects epidermal and dermal $CD8^+$ T cells from the same patient.

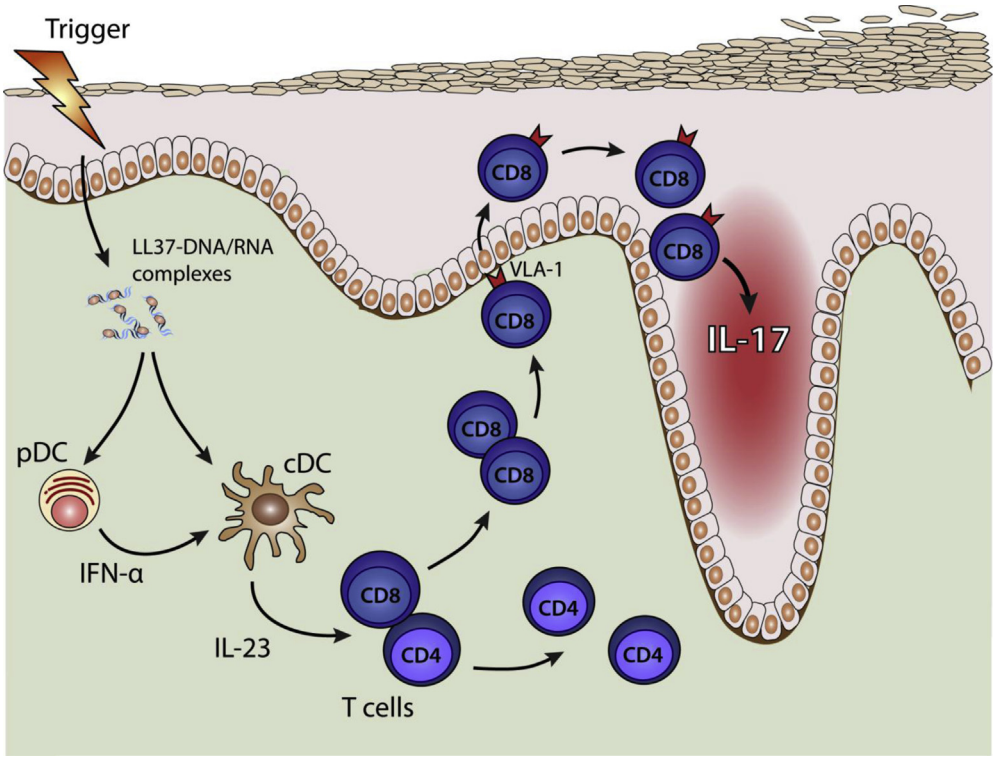

FIG E4. The role of $CD8^+$ T cells in psoriasis immunopathogenesis. Environmental triggers (eg, skin injury, known as Koebner phenomenon) induce the expression of LL37 by keratinocytes, which forms complexes with self-DNA/RNA released by dying cells. These complexes activate skin-infiltrating plasmacytoid dendritic cells (pDCs) to produce IFN-α, which in turn—together with LL37-RNA complexes—promotes maturation and activation of conventional dendritic cells producing IL-23. This leads to expansion and activation of autoreactive $CD8^+$ T cells, as well as $CD4^+$ T cells, in the dermis. Although $CD4^+$ T cells remain principally within the dermis, activated $CD8^+$ T cells acquire expression of very late antigen (VLA)-1 and migrate into the epidermis. Subsequently, potentially upon recognition of autoantigens on keratinocytes via MHC-I, intraepidermal $CD8^+$ T cells release IL-17, which is critically involved in psoriatic inflammation and its pathogenesis.

TABLE E1. Cellular and histologic changes over time during psoriasis development

Experiment depicted in Fig 1	Day 0	Day 7	Day 21	Day 35
Dermal T cells	3.22 (0.71)	16.08 (3.51)	22.30 (8.66)	6.65 (2.11)
Epidermal T cells	0.23 (0.08)	0.33 (0.24)	10.50 (1.62)	16.32 (1.68)
Papillomatosis index	1.507 (0.06)	1.598 (0.04)	2.529 (0.28)	2.978 (0.32)
Ki-67	16.67 (3.83)	11.67 (1.70)	56.39 (9.91)	72.78 (29.51)

Mean and SEM values of experimental data depicted in Fig 1, *A* and *B*. Mean (± SEM) values of dermal and epidermal T cells, papillomatosis index, and Ki-67 positive keratinocytes in skin samples upon xenotransplantation in the AGR mouse model at indicated time points. Values correspond to the data depicted in Fig 1, *A* and *B*.